Research Article

Halophytes: Gourmet food with nutritional health benefits?

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Abstract

Although little is known about their nutritional composition, Sarcocornia perennis subsp. perennis, S. perennis subsp. alpini and Salicornia ramossissima (Salicorniaceae) as well as Arthrocnemum macrostachyum (Amaranthaceae) are consumed in gourmet cuisine. In spite of belonging to different families, these halophytes share morphological and organoleptic characteristics. This work explored the nutritional properties and the antioxidant potential of these species using five integrative methods. All species had a nutritional profile suitable for human consumption with high levels of protein (5.20–13.2 g/100 g dw) and n-3 polyunsaturated fatty acids (FA), particularly α-linolenic acid (19.3–25.9% of total FA), and low concentration of toxic metals (below the limits imposed by the European Commission). These halophytes are also a good source of minerals, particularly sodium (64.1–109 mg/g dw), and S. ramossissima is an excellent source of manganese (204 μg/g dw). However, due care should be taken not to exceed the legal limits for sodium ingestion. These plants showed also significant antioxidant potential, with high radical scavenging activity (RSA), iron reducing power and total phenolics content (20.5–49.2 mg GAE/g). A. macrostachyum had the highest RSA (IC50 = 0.84 mg/mL; IC50,NO = 0.60 mg/mL), and iron reducing potential (IC50 = 0.84 mg/mL) along with high levels of α- and γ-tocopherol (8.74 and 4.71 mg/100 g dw, respectively).

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1. Introduction

Halophytes are traditionally consumed for their organoleptic and/or medicinal properties (Davy et al., 2001; Ventura and Sagi, 2013). The Salicornia L. (annual) and Sarcocornia L. (perennial) genera can be found in coastal salt marshes from the Arctic to the Mediterranean, and produce succulent shoots, which are highly appreciated in gourmet cuisine due to their salty taste (Ventura et al., 2011). In Europe, the young fleshy tips of different species of these genera are commercialized with the name ‘Samphire’ or ‘Sea asparagus’ (Ventura and Sagi, 2013).

As their salt tolerance and resistance to pests and diseases enables their cultivation in marginal or salinized soils using sea- or brackish water instead of freshwater (Glenn et al., 1999; Díaz et al., 2013; Ventura and Sagi, 2013), interest in halophyte plants has grown in recent years. The use of halophytic cash crops that can be domesticated through conventional breeding programmes aiming to improve their productivity and salt tolerance is therefore a promising approach for sustainable agriculture in marginal environments. In fact, biomass yields of halophytes can be similar to those of conventional crops, even when irrigated with seawater (Glenn et al., 1999; Ventura et al., 2011).

Arthrocnemum macrostachyum L. (glauccous glasswort) is a perennial C3 shrub with a morphology very similar to that of the Salicorniaceae. Overall, these species are common in salt marshes from Asian, European and North African countries, presenting interesting nutritional profiles with high levels of minerals, vitamin C and β-carotene (Glenn et al., 1999; Lu et al., 2010; Redondo-Gómez et al., 2010; Ventura et al., 2011; Essaidi et al., 2013). In addition, A. macrostachyum is rich in polyunsaturated fatty acids (PUFA) and is also a potential source of antioxidants (El-Wahab et al., 2008; Custódio et al., 2012; Rodrigues et al., 2014). Local producers and/or collectors all over Europe usually sell a mix of Salicorniaceae species due to incorrect taxonomical identification (Kadereit et al., 2007). Despite the relevance of these species for sustainable agriculture and of the Salicornia and Sarcocornia genera for gourmet cuisine a complete nutritional profile of these halophytes has never been established (Glenn et al., 2013). Hence, this work reports a comprehensive and comparative evaluation of

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the nutritional profile and in vitro antioxidant activity of four edible halophytes collected in southern Portugal (Algarve). The suitability of using these plants as food and as sources of natural antioxidants is discussed.

2. Material and methods

2.1. Plant material

Adult plants were collected in May 2010 in two different regions of the South of Portugal (Algarve): Castro Marim (S. ramosissima, S. perennis subsp. alpini and S. perennis subsp. perennis) and Praia de Faro (A. macrostachyum). Fig. 1 shows the location of sampling sites and the respective GPS coordinates. Dr Manuel J. Pinto (National Museum of Natural History, Botanical Garden, University of Lisbon, Portugal) was responsible for the taxonomical classification. At least 15 individuals of each species were randomly collected to ensure a representative sample. For proximate composition, minerals and antioxidant analysis, leaves were dried in an oven at 40 °C for 4 days, milled and stored at −20 °C prior to testing. The biomass was lyophilized for the determination of the FA profile and quantification of pigments and vitamins.

2.2. Nutritional profile

2.2.1. Proximate composition

Crude protein content (N × 6.25) was estimated by the macro-Kjeldahl method as described by Uslu et al. (2013); crude fat was determined gravimetrically by a modified protocol of the Bligh & Dyer method involving the homogenization of the dried biomass in a mixture of chloroform, methanol and water (2:2:1) using an IKA Ultra-Turrax disperser (IKA-Werke GmbH, Staufen, Germany), as described in Pereira et al. (2011); ash content was determined by incineration at 515 ± 15 °C for 5h. Neutral-detergent fibre (NDF) was analysed on a Fibretherm FT 12 Fibre Analyser (C. Gerhardt GmbH & Co, Koenigswinter, Germany) according to the AOAC Official Method 2002.04. Results are expressed as g per 100 g of dry weight (dw).

2.2.2. Fatty acids (FA) profile

FA were extracted from lyophilized rather than dried plants, since the drying process may alter FA composition. Extracted FA were transesterified to the corresponding FA methyl ester (FAME) and analysed by GC–MS as described in Pereira et al. (2012). Briefly, lyophilized samples were derivatised with a solution of methanol and acetyl chloride (20:1 w/w) for 1 h at 100 °C. After cooling, distilled water was added and the organic phase removed and dried with anhydrous sodium sulphate. FAME were analysed on an Agilent GC–MS (Agilent 6890 Network GC System, 5973 Inert Mass Selective Detector, Agilent Technologies, Santa Clara, CA, US) equipped with a DB5–MS capillary column (25 × 0.25 mm internal diameter, 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA, US). The oven temperature programme was: 60 °C (1 min), 30 °C min⁻¹ to 120 °C, 5 °C min⁻¹ to 250 °C, and 20 °C min⁻¹ to 300 °C (2 min). Identification and quantification of FAME (total ion mode) were performed by comparing the retention times of biodiesel samples with an external standard (Supelco 37 Component FAME Mix, Sigma-Aldrich Co. LLC, St. Louis, MO, US) and further confirmed by comparison of the MS spectra. For quantification purposes, a separate calibration curve was generated for each of the FAME in the standard. Results were expressed as percentage of total FAME content.

Fig. 1. Location and GPS coordinates of the sampling sites. Salicornia ramosissima, Sarcocornia perennis subsp. alpini and Sarcocornia perennis subsp. perennis were collected in Castro Marim and Arthrocnemum macrostachyum in Faro.
The atherogenicity and thrombogenicity indexes (IA and IT, respectively), used for the estimation of lipid quality, were calculated using the following equations (Ulbricht and Southgate, 1991):

\[
IA = (C12:0 + 4 \times C14:0 + C16:0) / [\Sigma MUFA + \Sigma (n-3) PUFA + \Sigma (n-6 PUFA)]
\]

\[
IT = (C14:0 + C16:0 + C18:0) / [0.5 \times \Sigma MUFA + 0.5 \times \Sigma (n-6 PUFA) + 3 \times (n-3 PUFA) + \Sigma (n-3 PUFA) / [\Sigma (n-6 PUFA)]
\]

where \(\Sigma MUFA, \Sigma (n-3 PUFA)\) and \(\Sigma (n-6 PUFA)\) are the sum of mono-unsaturated, n-3 and n-6 PUFA contents, respectively.

### 2.2.3. Mineral content

Three replicates of about approximately 300 mg each were microwave digested (Ethos Touch, Milestone Srl, Sorisole, Italy) in high-pressure Teflon vessels with 6 mL of supra-pure HNO\(_3\) (65%; Fluka, Sigma-Aldrich Co. LLC, St. Louis, MO, US), 1 mL of HClO\(_4\) (p.a. 70%; Riedel-de Haën, Honeywell, Morris Plains, NJ, US), and 1 mL of supra-pure H\(_2\)O\(_2\) (30%, Merck KGA, Darmstadt, Germany). A procedural blank was prepared and included in each digestion batch of 10 samples. Minerals were analysed by atomic absorption spectrometry-AAS (AGC Avanta Sigma, GBC Scientific Equipment Pty Ltd., Dandenong, Vic., Australia) provided with a deuterium background correction. The accuracy of the analytical procedure was assessed by the analysis of certified reference material BCR60 (Lagarisiphon major). Procedural blanks always accounted for less than 1% of the metal concentrations in samples. Results were expressed per g of dry weight (dw).

### 2.2.4. Tocopherols, total carotenoids and liposoluble pigments

Tocopherols were analysed immediately after freeze-drying following the procedure described by Bandarra et al. (2003). The extraction was carried out with 0.5 g of freeze-dried material, 2 mL of ethanol and 10 mg of ascorbic acid. The mixture was swirled for 2 min and 3 mL of n-hexane added. After a second swirling of the mixture for 2 min the sample was sonicated for 20 min in an ultrasonic bath to break the cell walls. After this treatment 2 mL of distilled water was added and the mixture stirred for 1 min followed by centrifugation for 10 min at 2150 \( \times \) g. The organic phase was recovered, filtered and dried with anhydrous sodium sulphate. The pellet was re-extracted twice with 1 mL and 0.5 mL of n-hexane respectively, but without ultrasonic treatment and water addition. The organic phases were pooled and a 20 \( \mu \)L aliquot was injected in a HPLC (JASCO model 980, JASCO, Easton, MD, US) equipped with an automatic injector and a fluorescent detector (JASCO Model FP-1520, JASCO, Easton, MD, US; \( \lambda_{ex} = 290 \) nm and \( \lambda_{em} = 324 \) nm). The separation was carried out in a Lichrosorb Si 60-5 (250 \( \times \) 3 mm i.d.) Chrompack column (Chrompack Inc., Raritan, NJ, US) protected by a silica pre-column S2-SS (10 \( \times \) 2 mm i.d.). The mobile phase was a mixture of n-hexane and isopropanol (99.3:0.7; v:v) degassed in the pump and eluted at a constant flow of 1 mL min\(^{-1}\). Tocopherol isomers were identified by comparison with retention times of standard references from Sigma (Sigma-Aldrich Co. LLC, St. Louis, MO, US) and the quantification made using the software Borwin 1.2. Total carotenoids were determined after extraction with acetone, and quantified spectrophotometrically as described by Uslu et al. (2013). Contents of chlorophyll \( a \) and \( b \) were determined according to Nagata and Yamashita (1992) using the following equations and further expressed in mg/100 g dw.

\[
\text{chlorophyll } a \ (\text{mg}/100 \text{ mL}) = 0.999 \times A_{663} - 0.0989 \times A_{645}
\]

\[
\text{chlorophyll } b \ (\text{mg}/100 \text{ mL}) = -0.328 \times A_{663} + 1.77 \times A_{645}
\]

### 2.3. Antioxidant activity

#### 2.3.1. Preparation of the extracts

Extracts were prepared by mixing the dried biomass with absolute ethanol (1:40, w/v), using a disperser IKA T10 B UltraTurrax (IKA-Werke GmbH, Staufen, Germany) for 2 min for cell disruption, as described in Rodrigues et al. (2015). Tubes were vortexed for 1 min at room temperature (RT, 20 °C) and centrifuged (1000 \( \times \) g, 10 min). Extractions were repeated twice, the supernatants combined, filtered (Whatman no. 4, GE Healthcare, Chicago, IL, US) and dried under vacuum. The dried extracts were resuspended in absolute ethanol to a final concentration of 10 mg/mL and stored at –20 °C. Different concentrations were prepared from the stock solution (0.25–10 mg/mL) and tested for antioxidant activity, as described below.

#### 2.3.2. Radical scavenging activity (RSA) on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO) radicals

RSA on the DPPH free radical was evaluated according to the method described in Moreno et al. (2006). The absorbance was measured at 515 nm (Biotek Synergy 4, BioTek Instruments, Winooski, VT, US) and RSA was calculated as percentage of inhibition relative to a control containing ethanol in place of the sample. Butylated hydroxytoluene (BHT) was used as a positive control. The NO scavenging activity was evaluated according to Ho et al. (2010). Absorbance was read at 546 nm (Biotek Synergy 4, BioTek Instruments, Winooski, VT, US) and ascorbic acid was used as positive control. The medium inhibitory concentrations (IC\(_{50}\)) were calculated only for those extracts displaying activities higher than 50% when tested at the concentration of 10 mg mL\(^{-1}\).

#### 2.3.3. Ferric reducing power activity (FRAP)

The ability of the extracts to reduce Fe\(^{3+}\) was assayed by the method described in Megías et al. (2009). Absorbance was measured at 700 nm (Biotek Synergy 4, BioTek Instruments, Winooski, VT, US). Increases in the absorbance of the reaction mixture indicate increased ferric reducing power. BHT was used as a positive control. The IC\(_{50}\) values were calculated only for those extracts displaying activities higher than 50% when tested at the concentration of 10 mg mL\(^{-1}\).

#### 2.3.4. Metal chelating activity on iron and copper

Iron chelating activity (ICA) was determined by measuring the formation of the Fe\(^{2+}\)-ferrozine complex (Megías et al., 2009). Change in colour was measured in a microplate reader at 562 nm (Biotek Synergy 4, BioTek Instruments, Winooski, VT, US). Copper chelating activity (CCA) was determined using pyrocatechol violet (PV; Megías et al., 2009). The change in colour of the solution was measured at 632 nm using a microplate reader (Biotek Synergy 4, BioTek Instruments, Winooski, VT, US). The synthetic metal chelator EDTA was used as a positive control for both metals. The IC\(_{50}\) values were calculated only for the extracts with activities higher than 50% when tested at the concentration of 10 mg mL\(^{-1}\).

#### 2.3.5. Total polyphenols (TPC) and flavonoids (TFC) content

TPC was determined by the Folin-Ciocalteu assay (Velioglu et al., 1998). The absorbance was measured at 725 nm on a microplate reader (Biotek Synergy 4, BioTek Instruments, Winooski, VT, US) and results were expressed as mg of Gallic acids equivalents (GAE) per g of dried extract. TFC was estimated by the AlCl\(_3\) colorimetric assay adapted to 96-well microplates (Zou et al., 2011). Absorbance was measured at 510 nm and catechin was...
used as standard. Results were expressed as milligrams of catechin equivalents per gram of dried sample (mg CE/g dw).

2.4. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM), and experiments were conducted at least in triplicate. Significant differences were assessed by analysis of variance (ANOVA). When parametricity of data could not be assumed the non-parametric test Duncan’s New Multiple Range Test was used. SPSS statistical package for Windows (release 15.0, SPSS Inc.) was used. The IC₅₀ values were calculated by sigmoidal fitting of the data in the GraphPad Prism v. 5.0 software.

3. Results and discussion

3.1. Nutritional profile

3.1.1. Proximate composition

Overall, moisture, fat and ash contents of the Salicorniaceae included in this work did not vary significantly when compared to each other (Table 1) and were similar to those of other edible halophytes, such as Sporobolus virginicus and Sueda glauca (Alhadrami et al., 2010; Díaz et al., 2013). The main differences were found between the total protein contents of A. macrostachyum, Salicornia and Sarcocornia. The first species contained significantly higher amounts (p < 0.05) of this class of biomolecules than the last two of the three, thus being a better source of protein.

Halophytes, in general, have higher ash contents than other edible plants (Borah et al., 2009). Ash content is related to the total mineral concentration, and thus the high ash levels observed in these Salicorniaceae are most probably related to the saline environment in which they grow and their ability to retain minerals (Redondo-Gómez et al., 2010; Díaz et al., 2013). Halophytes have developed a set of characteristics enabling them to withstand high levels of salinity. One of them is the accumulation and compartmentalization of compatible solutes and ions for osmotic adjustment. In addition, they are able to regulate transpiration and accumulate essential nutrients like K in the presence of high Na and Cl concentrations (Flowers et al., 2010). As a result, halophyte plants are valuable sources of essential minerals. The minerals profile of these halophytes is presented and discussed below in the micronutrients section.

Interestingly, halophytes tend also to contain high fibre contents (Díaz et al., 2013). In this study, the Neutral Detergent Fibre (NDF) was determined as an estimate of the amounts of fibres such as cellulose, hemicellulose and lignin as well as cutin and tannins. Although NDF is generally used for the assessment of feed quality, it is considered to be a reliable analytical tool for the estimation of the insoluble portion of dietary fibre in food (Dhingra et al., 2012). A. macrostachyum and S. perennis perennis displayed the highest contents of insoluble fibre, higher than those of most vegetables, but similar to the levels found in beetroot or eggplant (Dhingra et al., 2012) or in other halophytes like Bassia hyssopiphipolia (Díaz et al., 2013). Although showing lower fibre contents, S. perennis alpini and S. ramosissima can still be considered as fibre-rich vegetables. Their fibre contents were similar to those measured in other Salicornia species such as Salicornia bigelovii (Díaz et al., 2013). A daily intake of 7 g of fibre of cereal or vegetable origin was considered enough to significantly reduce the risk of cardiovascular and coronar heart disease (Threapleton et al., 2013). The intake of 100 g of edible portions (wt weight) of A. macrostachyum, S. perennis alpine, S. perennis perennis and S. ramosissima would thus cover 92%, 48%, 71% and 50%, respectively, of such recommended daily dose. Overall, the studied halophytes can be considered to be valuable nutritional and healthy food, rich in minerals, fibres and proteins and poor in fat.

3.1.2. FA profile

In general, the profiles here presented were in agreement with those described by other authors for other species belonging to the Salicornia and Sarcocornia genera (Table 2; Ventura et al., 2011; Ksouri et al., 2012; Essaidi et al., 2013). The four species had a rather saturated FAME profile with the sum of saturated FA (SFA) always higher than 50%. Such saturated profiles are common in halophytic plants and are probably related to salt tolerance mechanisms. For example, the vascular membranes of S. maritime contain high contents of saturated FA, which seem to contribute to decreased membrane permeability to NaCl (Glenn et al., 1999). In addition, Mesembryanthemum crystallinum exposed to different Cd concentrations exhibited increasing FA saturation levels that correlated positively with metal concentration (Nouairi et al., 2006). The monounsaturated FA (MUFA) were present in very low amounts. Although just a few studies can be found regarding the distribution of FA amongst plant organs, Weete et al. (1970) reported a significantly higher content of MUFA in the seeds of Salicornia bigelovii than in other tissues. From a nutritional standpoint, however, unsaturated FA profiles with high abundances of n-3 FA such as eicosapentaenoic (EPA; C20:5), docosahexaenoic (DHA; C22:6) and α-linolenic (ALA; C18:3n-3) acids are usually preferred, since these PUFA have several important biological activities, such as anti-bacterial, anti-inflammatory and antioxidant, linked to their capability to prevent cardiac diseases and to inhibit tumour progression (Pereira et al., 2012). EPA and DHA, however, are typical of marine organisms, such as microalgae, bacteria, fish and shellfish, but are rarely found in terrestrial plants (Robert, 2006). Accordingly, the PUFA profile of the studied plants showed a predominance of ALA and linolenic acid (LA). A. macrostachyum had the most interesting profile with a PUFA content of 47.0%, significantly higher (p < 0.05) than those of the Salicorniacae. The n-3 PUFA ALA was the most abundant FA in the profile of A. macrostachyum (25.9%), a proportion significantly higher (p < 0.05) than those of other species (Table 2). This feature resulted in better PUFA/SFA ratios. Considering the role of PUFA in cardiac diseases, the indexers of atherogenicity (IA) and thrombogenicity (IT) are commonly used to interpret and predict the health benefits associated with the consumption of several foods. IA indicates the relationship between the main classes of saturated

<table>
<thead>
<tr>
<th>Contents</th>
<th>Arthrocnemum macrostachyum</th>
<th>Sarcocornia perennis alpini</th>
<th>Sarcocornia perennis perennis</th>
<th>Sarcocornia ramosissima</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>81.2 ± 1.0⁴</td>
<td>84.0 ± 1.4⁴</td>
<td>85.8 ± 0.5⁴</td>
<td>84.5 ± 0.2⁴</td>
</tr>
<tr>
<td>Protein</td>
<td>13.2 ± 0.2⁴</td>
<td>8.10 ± 0.06⁴</td>
<td>6.90 ± 0.17⁴</td>
<td>5.20 ± 0.29⁴</td>
</tr>
<tr>
<td>Fat</td>
<td>2.83 ± 0.11⁴</td>
<td>1.20 ± 0.09⁴</td>
<td>2.25 ± 0.05⁴</td>
<td>1.87 ± 0.18⁴</td>
</tr>
<tr>
<td>NDF</td>
<td>34.3</td>
<td>20.8</td>
<td>34.1</td>
<td>22.5</td>
</tr>
<tr>
<td>Ash</td>
<td>31.6 ± 0.4⁴</td>
<td>30.7 ± 1.1⁴</td>
<td>23.3 ± 0.3⁴</td>
<td>29.2 ± 0.6³</td>
</tr>
</tbody>
</table>

NDF – Neutral detergent fibre. In each row, different letters mean significant differences (p < 0.05).
and unsaturated FA. Since saturated FA are considered to be pro-atherogenic and unsaturated FA anti-atherogenic, a low IA ratio is desirable (Garaffo et al., 2011). The IT indicates the tendency to form clots in the blood vessels and is defined as the relationship between the pro-thrombogenic (saturated FA) and the anti-thrombogenic (MUFA, n-3 and n-6 PUFA). Therefore, a low value of IT is also desirable (Garaffo et al., 2011). The IA and IT values for A. macrostachyum and the other Salicorniae are shown in Table 2. Considering these indexes, A. macrostachyum is the most likely to provide the best health benefits. Interestingly, the IA and IT values presented by all plants assessed in this study are very similar to those calculated for tuna, a fish known by its “healthy” supply of unsaturated FA (Garaffo et al., 2011). Such low values of IT and IA reflect the relatively high content in EPA and DHA found in tuna. In the studied plants, however, these indexes are mostly due to the high proportion of ALA of their FA profiles. ALA has several beneficial effects on human health: i) it is a precursor of EPA and DHA and evidence shows that increased ALA consumption is associated to higher ALA, EPA, docosapentaenoic acid (DPA; 22:5n-3) and, to a lesser extent, DHA tissue contents; ii) it may reduce arachidonic acid (ARA; 20:4n-6) levels by competing with LA for the same metabolic enzymes (e.g. responsible for the elongation of n-6 FA); iii) it can reduce the levels of serum triglycerides; iv) its anti-inflammatory properties may help in the prevention of primary and secondary coronary artery disease by a different mechanism than that of EPA and DHA; and v) it may display a positive effect on the recovery of a central nervous system injury (Barceló-Coblijn and Murphy, 2009).

### Table 3

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Arthrocnemum macrostachyum</th>
<th>Sarcocornia perennis alpini</th>
<th>Sarcocornia perennis perennis</th>
<th>Sarcocornia ramosissima</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mg/g)</td>
<td>109 ± 3.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>64.3 ± 0.9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>64.1 ± 0.9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>89.9 ± 0.5&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>K (mg/g)</td>
<td>15.8 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>10.3 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>13.9 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>8.92 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ca (mg/g)</td>
<td>3.30 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.63 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.34 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.86 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Mg (mg/g)</td>
<td>5.92 ± 0.11&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.03 ± 0.04&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.73 ± 0.08&lt;sup&gt;e&lt;/sup&gt;</td>
<td>9.43 ± 0.08&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fe (mg/g)</td>
<td>0.42 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.28 ± 0.05&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.82 ± 0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.53 ± 0.02&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mn (mg/g)</td>
<td>28.6 ± 1.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>65.2 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>314 ± 0.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>204 ± 4.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zn (mg/g)</td>
<td>31.0 ± 0.9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>25.2 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>16.9 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>68.7 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cr (mg/g)</td>
<td>2.08 ± 0.10&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.92 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.67 ± 0.06&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.24 ± 0.05&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pb (mg/g)</td>
<td>nd&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.31 ± 0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.45 ± 0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.45 ± 0.02&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ni (mg/g)</td>
<td>0.49 ± 0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.64 ± 0.03&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.91 ± 10&lt;sup&gt;10&lt;/sup&gt;</td>
<td>2.32 ± 0.10&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cd (mg/g)</td>
<td>nd&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.39 ± 0.00</td>
<td>nd&lt;sup&gt;f&lt;/sup&gt;</td>
<td>nd&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

nd – Not detected. In each row, different letters mean significant differences (p < 0.05). *LOD – 0.03 μg/g. **LOD – 0.11 μg/g.
A distinctive feature of these plants is their particularly high Na content (64.1–109 mg/g dw), higher than that of some food products commonly accepted as good Na sources, such as seaweeds (1.46–39.6 mg/g dw; El-Said and El-Sikaily, 2013). Although Na is an essential nutrient, its consumption in excess has been linked to several pathologies including hypertension and cardiovascular diseases (Kotchen et al., 2013). Thus, the World Health Organization (WHO) recommends that the daily intake of sodium should not exceed 2000 mg. Considering edible portions of these plants, a consumption of 100 g of fresh tips of *A. macrostachyum*, *S. perennis alpini*, and *S. ramosissima* would represent intakes of 2049, 1029, 910 and 1393 mg of Na, respectively. Hence, due care must be taken so that the maximum allowed daily dose recommended by WHO is not exceeded. The levels of other minerals are similar to those found in different edible wild plants and these halophytes can be considered to be good nutritional sources of minerals such as K (8.92–15.8 mg/g dw), Ca (2.34–4.86 mg/g dw) and Fe (0.42–1.53 mg/g dw; Aberoumand and Deokule, 2009). Mn had the widest variation amongst these species and its concentrations ranged between 28.6 (A. macrostachyum) and 204 µg/g dw (S. ramosissima). Plants belonging to the genus Salicornia appear to be particularly resistant to Mn and reports of elevated concentrations in Salicornia species are frequently found in the literature (Ventura et al., 2013). Given the high Mn levels, around 60 g of fresh tips of *S. ramosissima* would be able to meet the daily intake of 2 mg of Mn recommended by the WHO without exceeding the maximum amount of sodium recommended. In general, the levels of toxic metals (Cr, Pb, Ni and Cd) were below legislated values (0.3 mg/kg ww for Pb and 0.2 mg/kg ww for Cd; EC Regulation 1881/2006) and not markedly different from those observed in *S. fruticosa* from the Ria Formosa or from the Guadiana and Tagus estuaries (Caetano et al., 2008; Moreira da Silva, 2008). Cd, in particular, was only detected in *S. perennis alpini* and at very low concentration. Nonetheless, when located in polluted saltmarshes, halophytes have the ability to accumulate metals like Zn, Cr, Pb, Ni and Cd (Caetano et al., 2008). The highest concentrations of these metals are, however, usually found in the roots, as determined for the species *S. fruticosa*, *S. ramosissima* and *A. macrostachyum*, and translocation to aboveground tissues is usually low (Caetano et al., 2008; Moreira da Silva, 2008; Redondo-Gómez et al., 2010). Conversely, Fe and Mn usually present higher translocation factors, due to their role in photosynthesis, and are therefore present in aboveground tissues at higher concentrations (Moreira da Silva, 2008).

### 3.1.4. Chlorophyll, carotenoid and tocopherol composition

The chlorophyll, total carotenoid and tocopherol composition of the studied halophytes is presented in Table 4. Carotenoids are a wide range group of natural products synthesized by photosynthetic organisms, yeasts and fungi, and are known for their antioxidant and immunomodulating properties (Bernal et al., 2011). *A. macrostachyum* had the lowest carotenoid level although the analysed species presented similar levels to that of *Salicornia prostrata* when exposed to high salinities, which is consistent with the summer conditions experienced in the Algarve region (Akcin and Yalcin, 2016). Although the amount of chlorophylls is not especially important in terms of nutritional profiling, it provides a measure of the green vegetable colour and is generally used by consumers as an estimate of vegetable senescence (Ventura et al., 2011). In this sense, the shoots of *A. macrostachyum* may be more attractive to consumers due to its higher amount of chlorophylls (sum of chlorophyll *a* and *b*). Tocopherols are fat-soluble antioxidants that play an important role in the prevention of lipid peroxidation by reactive oxygen species (Bernal et al., 2011). In this study, three tocopherol isoforms were analysed α-tocopherol being the prevailing isofrom. This distribution is common amongst halophytes in which α-tocopherol is generally the most abundant (Ksouri et al., 2012). *A. macrostachyum* had the highest content of α-tocopherol (8.74 mg per 100 g dw or 1.64 mg/100 g of edible portion). This concentration is similar to those of other raw vegetables such as red peppers, kale or broccoli (1.58, 1.54 and 1.62 mg/100 g of edible portion; USDA, 2015). Even though tocopherols are generally more abundant in seeds oil, in which they can reach concentrations of 569 mg kg⁻¹ of oil, the shoots of *A. macrostachyum* can be also a good source of those compounds, especially of γ-tocopherol (Davy et al., 2001; Ksouri et al., 2012). The level of this nutrient in *A. macrostachyum* (4.71 mg per 100 g dw) is similar to that found in rice bran, a particular good source of γ-tocopherol (2.09–4.1 mg per 100 g dw; Aguilar-Garcia et al., 2007). The elevated γ-tocopherol concentration is rather interesting. Even though most vitamin supplements contain the α isofrom, it has been shown that a combination of α- and γ-tocopherol has a more potent anti-inflammatory effect than the α isofrom alone (Reiter et al., 2007; Devaraj et al., 2008). In this context, *A. macrostachyum* appears to have a more balanced composition.

#### 3.2. Antioxidant activity

#### 3.2.1. Total phenolics (TPC) and flavonoids content (TFC)

Phenolic compounds are known as good radical scavengers, and play an important role in human health, since they are able to minimize the deleterious effects of oxidative stress. Amongst phenolic compounds, flavonoids are considered as dietary antioxidants with anti-inflammatory, antiviral, antibacterial and metal chelating activities (Gargouri et al., 2013). The TPC values here reported for the *Salicornia* and *Sarcocornia* species (Table 5) were lower than those found in *S. herbacea* or *S. perennis*, but similar to those of other halophytes such as *M. crystallinum* and *M. nodiflorum* (Ksouri et al., 2012; Essaidi et al., 2013; Gargouri et al., 2013). Contrary to TPC, the TFC of these halophytes was generally higher (Table 5) than those reported in the literature for the *Sarcocornia* and *Salicornia* genera (Gargouri et al., 2013). *A. macrostachyum*, however, had by far the highest contents (49.2 mg GAE/g dw and 41.9 mg CE/g dw, respectively), confirming its role as an important

### Table 4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Artemochromen macrostachyum</th>
<th>Sarcocornia perennis alpini</th>
<th>Sarcocornia perennis perennis</th>
<th>Salicornia ramosissima</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a</td>
<td>13.6 ± 3.0⁹</td>
<td>9.93 ± 0.7¹</td>
<td>8.26 ± 0.4⁶</td>
<td>13.8 ± 2.1²</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>14.7 ± 4.3¹</td>
<td>4.75 ± 0.6¹</td>
<td>6.52 ± 1.8⁶</td>
<td>7.76 ± 1.3³</td>
</tr>
<tr>
<td>Total carotenoids</td>
<td>210 ± 10⁴</td>
<td>290 ± 10⁴</td>
<td>280 ± 10⁴</td>
<td>290 ± 20³</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>8.74 ± 0.1⁹</td>
<td>2.01 ± 0.1¹</td>
<td>1.11 ± 0.1⁹</td>
<td>1.14 ± 0.07⁷</td>
</tr>
<tr>
<td>γ-tocopherol</td>
<td>4.71 ± 0.1¹</td>
<td>0.96 ± 0.06⁷</td>
<td>0.09 ± 0.01⁷</td>
<td>nd¹</td>
</tr>
<tr>
<td>δ-tocopherol</td>
<td>nd¹</td>
<td>nd¹</td>
<td>nd¹</td>
<td>0.77 ± 0.05</td>
</tr>
</tbody>
</table>

nd = Not detected. In each row, different letters mean significant differences (p < 0.05). ¹LOD = 0.07 mg/100 g.
Table 5

<table>
<thead>
<tr>
<th></th>
<th>Arthrocnemum macrostachyum</th>
<th>Sarcocornia perennis alpini</th>
<th>Sarcocornia perennis</th>
<th>Salicornia ramosissima</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC</td>
<td>49.2 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.7 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.5 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.0 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TFC</td>
<td>41.9 ± 4.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.3 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.9 ± 2.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>17.5 ± 2.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Radical Scavenging Activity and Ferric Reducing Power</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPPH</td>
<td>0.84 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.5 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.04 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.69 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NO</td>
<td>0.60 ± 0.09</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10</td>
</tr>
<tr>
<td>FRAP</td>
<td>0.84 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.55 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.77 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Metal Chelating Activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICA</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>4.38 ± 0.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.12 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCA</td>
<td>6.93 ± 0.91</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

DPPH – 1,1-diphenyl-2-picrylhydrazyl radical; NO – Nitric oxide radical; ICA – Iron chelating activity; CCA – Copper chelating activity; FRAP – Ferric reducing antioxidant power. These means were compared using the Tukey honestly significant differences test (p < 0.05). The DPPH (mg/mL) of the positive controls was as follows: DPPH (BHT – 0.07); NO (Ascorbic acid – 2.50); ICA (EDTA – 0.10); CCA (EDTA – 0.28).

source of antioxidant molecules. Although working with different extracts, Rodrigues et al. (2014) also identified A. macrostachyum as a good source of phenolics and flavonoids. Phenolic compounds, including flavonoids, are secondary metabolites that play different roles in the phytochemical and cellular mechanisms of plants, including pigmentation as well as resistance to pests, predators and oxidative stress. Halophytes live in extremely harsh environments with high salinities and UV radiation and these stressful conditions often lead to the production of radical oxygen species. Because they often display antioxidant properties, halophyte plants most likely produce phenolic compounds as additional components of their response to oxidative stress (Ksouri et al., 2008).

3.2.2. Radical scavenging activity (RSA) and ferric reducing antioxidant power (FRAP)

Given the different response of antioxidants to different radicals or oxidant sources, a single assay is generally not enough to assess the antioxidant activity of complex systems as plant extracts (Custódio et al., 2012). Therefore, the RSA of the ethanol extracts of the four halophytes was assessed by five complementary methods including their ability to scavenge the free radicals DPPH and NO, their power to reduce ferric ion and their capacity to chelate the transition metals iron and copper. Among the halophytes tested, A. macrostachyum had the best ability to scavenge DPPH and was the only species able to scavenge NO (Table 5). Moreover, the ability of A. macrostachyum to scavenge NO (IC<sub>50</sub> = 0.60 mg/mL) is almost 5-fold higher than that of ascorbic acid (IC<sub>50</sub> = 2.50 mg/mL). The antioxidant activity of extracts depends upon the extracted compounds, and in turn these vary according to the extraction solvent and conditions used. Do et al. (2014) reported higher antioxidant activity for the ethanol extract of the medicinal plant Limnophila aromatica compared to other solvents. As phenolic compounds have been described as powerful antioxidants (Ksouri et al., 2009), the high RSA is probably linked to the high TPC found in the extracts of the studied halophytes. Indeed, higher levels of phenolics found in A. macrostachyum (Table 5) would explain the its higher antioxidant potential. In addition, when compared to other halophytes, A. macrostachyum had also significantly higher contents of tocopherols (Table 4), a group of biochemicals known to have antioxidant properties (Bernal et al., 2011).

3.2.3. Metal chelating activity

The ability to chelate divalent ions such as iron and copper, decreasing their bioavailability, is important for the prevention of oxidative stress, since these metals may participate in oxyradical formation reactions (Megias et al., 2009). The ethanol extracts of the four halophytes had moderate metal chelating capacities. However, these extracts showed significant metal chelation selectivity, i.e., S. perennis perennis and S. ramosissima were able to chelate iron but not copper and A. macrostachyum chelated copper but not iron. Selective chelators with lower binding affinities may help to mobilize metal ions more efficiently without the adverse effects of most known metal chelators (Robert et al., 2015).

4. Conclusions

Although the species collected in this study were analysed separately, they are often consumed in mixtures that producers sell under the generic name “Salicornia”. This arises from the difficulty associated to the correct identification of the several Salicorniaceae species that often present weak morphological variation. Significant differences were found among the nutritional profiles of the different genera studied, but all species had nutritional profiles suitable for human consumption. A. macrostachyum had the most interesting nutritional profile and its remarkable content in phenolics and tocopherols justifies its use as succulent salty shoots in gourmet cuisine. Our results emphasize the importance of studying not only A. macrostachyum, but also species from the Salicornia and Sarcocornia genera for their contents in nutritional elements with possible benefits for human health.

Acknowledgements

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